

Original Article

Influence of phenotype conversion of epicardial adipocytes on the coronary atherosclerosis and its potential molecular mechanism

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Received August 6, 2015; Accepted October 11, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Objective: To investigate the phenotype conversion of epicardial adipocytes and its potential molecular mechanism during the occurrence and development of coronary atherosclerosis. Methods: A total of 30 health male New Zealand white rabbits were used. In experiment group (n=15), rabbits were fed with high fat food to establish atherosclerosis animal model; rabbits in control group (n=15) were fed with normal food. Results: At week 0, UCP-1 and PPAR γ mRNA expressions in EAT and sBAT were significantly higher than in eWAT, and leptin mRNA expression lower than (P<0.05). In experiment group, the mRNA expressions of UCP-1 and PPAR γ reduced gradually, but leptin mRNA increased progressively in EAT (P<0.05). UCP-1 expression reduced gradually, the newly generated blood vessels reduced significantly, but leptin and RAM11 increased gradually (P<0.05). The adipocyte volume in EAT increased gradually, but the adipocyte number reduced progressively (P<0.05). The number of mitochondria with multiple crests reduced gradually in EAT; IL-6 reduced the mRNA expressions of UCP-1 and PPAR γ in adipocytes of BAT in a dose dependent manner, but it increased the mRNA expressions of leptin and STAT3 (P<0.05). In the presence of IL-6, JSI-124 increased the mRNA expressions of UCP-1 and PPAR γ in adipocytes of BAT in a dose dependent manner, but it reduced the mRNA expressions of leptin and STAT3 (P<0.05). Conclusion: During the progression of atherosclerosis, there is a phenotype conversion of EAT from BAT to WAT, which further promotes the focal occurrence and development of atherosclerosis; IL-6 may activate JAK-STAT3 pathway to induce this conversion.

Keywords: Epicardiac adipose tissue, atherosclerosis, cellular phenotype, IL-6, STAT

Introduction

According to the adipocyte phenotype, adipose tissues in mammals can be divided into white adipose tissues (WAT) and brown adipose tissues (BAT). There are significant differences in the sites of distribution, cellular morphology and molecular markers between WAT and BAT. BAT mainly produce heat and consume energy via over-expressing UCP-1, which is helpful for the maintenance of energy and metabolism balances. However, WAT may secrete some adipokines (such as leptin and resistin) and pro-inflammatory cytokines to induce local or even systemic inflammatory reaction, resulting in or aggravating metabolic disease [1]. Epicardial adipose tissues (EAT) are close to the coronary artery and myocardium, which is an anatomic

advantage of EAT. In addition, EAT may secrete a variety of pro-inflammatory cytokines to facilitate the progression of coronary atherosclerosis. Our previous studies showed the EAT volume and pro-inflammatory cytokines secreted by EAT significantly affected the severity of coronary heart disease (CHD) and the stability of plaques [2, 3]. However, in the development of coronary atherosclerotic plaques, the change in adipocyte phenotype of EAT and its influence have never been reported. In this study, subscapularis BAT and epididymal WAT were employed as controls, the change in phenotype of EAT was investigated during the occurrence of coronary atherosclerosis, and the molecular mechanism underlying the pro-inflammatory cytokines induced conversion of EAT was also explored. Our findings may provide evidence for

Table 1. Primers used for real time qualitative PCR

Target gene	Forward (5'-3')	Reverse (5'-3')
β-actin	CGCAGAAACGACGACGAGATTGG	AGAACTTTGGGGACTTTGGGGAT
UCP-1	CACGGGGACGTACAATGCCT	GCTAGTATTCGTTTCTCACAAGGG
PPAR _γ	TGGGGATGTCTCATAATGCCA	TTCCTGTCAAGATCGCCCTCG
Leptin	GGGTCGTCGGTTTGGACTTCA	GTCGTTGGCTATTGGATCACATT
STAT3	GCTTTTGTCTGCAATGGAGTACGT	CCAGTTTCTTAATTTGTTGGCGAGT

Notes: UCP-1: including uncoupling protein 1; PPAR- γ : peroxisome proliferator-activated receptor- γ ; STAT3: Signal transducer and activator of transcription-3.

the pathogenesis of coronary atherosclerosis and present new clues for the therapy of coronary atherosclerosis.

Materials and methods

Materials

Animals: A total of 30 healthy male New Zealand white rabbits were used in this study and housed in cages for 1 week before experiments. In experiment group, animals (n=15) were fed with high fat diet (1% cholesterol, 5% egg yolk powder and 8% lard in normal food). In control group, animals (n=15) were fed with normal food.

Reagents: DMEM high glucose, fetal bovine serum (FBS), PBS buffer (Gibco, USA), dexamethasone (DEX), type II collagen, insulin, 3-isobutyl-1-methylxanthine (IBMX) (Sigma, USA) TRIzol (Invitrogen, USA), reverse transcription kit (Promega, USA), QuantiTect SYBR Green PCR MasterMix (Toyobo, Japan), STAT3 specific inhibitor JSI-124 (Calbiochem) and RT-PCR kit (TaKaRa) were used in the present study.

Methods

Blood collection: At 0, 8 and 12 weeks, rabbits were fasted for 12 h, and then 2 ml of venous blood was collected from the ear vein. Thirty minutes later, the plasma was collected and processed for the detection of TG, TC, LDL-C, HDL-C and hs-CRP. ELISA was employed to detect the IL-6, TNF- α , leptin, PAPP-A, adiponectin, sE-selectin and MMP-9 in the plasma.

Sample collection: At 0, 8 and 12 weeks, 5 rabbits were randomly selected from each group and fasted for 12 h. Then, animals were intraperitoneally anesthetized with pentobarbital sodium at 25 mg/kg and sacrificed. Under an aseptic condition, EAT, sBAT, eWAT and heart

were rapidly collected. For RT-PCR, adipose tissues were placed into liquid nitrogen and stored at -80°C; for immunohistochemistry, rabbits were perfused with 4% paraformaldehyde and tissues were further fixed in 4% paraformaldehyde (v:v 1:10).

Pathological examination and histochemistry: The heart was collected and cut into 5- μ m

consecutive sections at the level of proximal part of anterior descending artery, following by Masson staining and subsequent evaluation of plaques. Adipose tissues were embedded in paraffin and cut into 5- μ m consecutive sections. HE staining was performed to observe the morphology of adipocytes, and immunohistochemistry was done for UCP1, leptin, CD31 and RAM11 (rat anti-rabbit macrophage antibody 11).

Transmission electron microscopy (TEM): Adipose tissues were cut into 1-mm³ blocks and fixed in 2.5% glutaraldehyde for 3 h, followed by processing for TEM. The morphology and amount of mitochondria in the adipose tissues were observed and determined, respectively.

RT-PCR: Total RNA was extracted from adipose tissues according to manufacturer's instructions and reverse transcribed into cDNA. cDNA served as a template for RT-PCR, and primers used in RT-PCR are shown in **Table 1**. RT-PCR was performed in 25 μ l of mixture and conditions were as follows: predenaturation at 95°C for 10 min, a total of 44 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 40 s, and reading at 80°C for 5 s, and melting curve was obtained at 72-94°C. β -actin served as a loading control.

Separation and culture of preadipocytes: EAT was collected from control group and placed into pre-cold PBS. The surface connective tissues and blood vessels were removed. Adipose tissues were cut into blocks (1 mm $\times$ 1 mm $\times$ 1 mm) which were then transferred into sterilized 50-ml centrifuge tubes, followed by addition of digestion solution (1.5 g/L type II collagenase, 20 g/L bovine serum albumin and 1.2 g/L HEPES). Incubation was done at 37°C for 1 h at constant shaking. After the adipose tissues were digested, the mixture was filtered through a 30-mesh filter. The resultant mixture was

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Table 2. Blood lipid and inflammatory cytokines at different time points ($\bar{X} \pm s$)

	Control			Experiment		
	0 week	8 weeks	12 weeks	0 week	8 weeks	12 weeks
TG (mmol/L)	0.94±0.13	0.96±0.11	0.93±0.07	0.95±0.08	1.86±0.19*	1.99±0.12*. [#]
TC (mmol/L)	3.25±0.62	3.22±0.48	3.26±0.51	3.28±0.53	24.65±1.63*	26.27±1.94*. [#]
LDL-C (mmol/L)	2.29±0.22	2.35±0.19	2.31±0.31	2.33±0.25	21.17±2.03*	24.31±1.98*. [#]
HDL-C (mmol/L)	1.61±0.31	1.59±0.29	1.64±0.32	1.63±0.34	1.29±0.18*	1.08±0.11*. [#]
IL-6 (pg/ml)	18.56±7.23	19.12±8.37	18.88±7.95	18.92±7.25	179.97±32.60*	626.85±76.78*. [#]
TNF-α (pg/ml)	475.54±20.03	467.49±19.54	469.32±19.21	464.73±19.94	625.11±16.32*	754.21±37.74*. [#]
Leptin (pg/ml)	91.86±7.03	92.56±7.36	93.51±8.01	92.02±7.12	143.09±15.94*	152.71±22.61*. [#]
MMP-9 (ng/ml)	56.92±7.96	57.23±8.72	57.58±8.42	57.11±8.16	125.21±22.41*	152.26±11.01*. [#]
PAPP-A (ng/ml)	0.10±0.04	0.12±0.05	0.11±0.02	0.11±0.05	0.55±0.05*	0.73±0.02*. [#]
sE-selectin (ng/ml)	59.81±11.07	61.35±14.35	60.78±13.43	60.31±14.67	101.45±17.40*	135.60±23.23*. [#]
Adiponectin (ng/ml)	1.70±0.03	1.69±0.07	1.68±0.04	1.68±0.07	0.72±0.06*	0.23±0.15*. [#]

Notes: TG: triglycerides; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high sensitive C-reactive protein; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; MMP-9: matrix metalloproteinase-9; PAPP-A: pregnancy associated plasma protein-A. sE-selectin: soluble type of E-selectin. *p<0.05 vs. 0 week, [#]p<0.05 vs. 8 weeks.

transferred into a new 50-ml centrifuge tube which was allowed to stay on ice for 15-30 min. When different layers formed in the tube, the middle layer was transferred and filtered through a 300-mesh filter, and the filtrate was transferred into a new sterilized centrifuge tube, followed by centrifugation at 700 rpm/min for 10 min. The supernatant was removed, and the sediments were mixed with red cells lysis buffer, followed by incubation at room temperature for 10 min. After centrifugation at 700 rpm/min for 10 min, the supernatant was removed, and the sediment was washed with PBS by centrifugation at 700 rpm/min for 10 min. The sediment was re-suspended in DMEM and cells were seeded into 6-well plates, followed by incubation at 37°C in an environment with 5% for 6 h. The non-adherent cells were removed by washing with PBS, and cells were further maintained. The medium was refreshed once every 3 days, and cell morphology was observed under a microscope.

Induced differentiation of preadipocytes: Above preadipocytes were maintained in DMEM containing 10% FBS and 20 nmol/L insulin. When cell confluence reached about 100%, the medium was refreshed with adipogenic medium (brown adipose culture medium including 0.5 mmol/L IBMX and 0.5 μmol/L DEX), followed by incubation for 2 d. Then, the medium was refreshed with adipose culture medium. At 3-4 d after induced differentiation, lipid droplets were present, and cells became mature at 6-8 d.

Oil red staining of adipocytes: When the adipocytes became mature, the medium was removed, and cells were fixed in 10% formalin at room temperature for 1 h. Then, cells were stained with oil red solution (0.5 g of oil red O in 100 ml of isopropanol) for 30 min. After washing, cells were observed under a light microscope.

IL-6 treatment: Preadipocytes (2×10⁶/ml) were seeded into 6-well plates and induced to differentiate into adipocytes. The mature adipocytes were treated with IL-6 at different concentrations (0, 5, 10, 20, 50 and 100 ng/ml) in DMEM/F12 for 24 h at 37°C in an environment with 5% CO₂. Total RNA was extracted, and RT-PCR was employed to detect the mRNA expressions of UCP-1, leptin, PPARγ and STAT3.

Treatment with STAT3 specific blocker JSI-124: Preadipocytes (2×10⁶/ml) were seeded into 6-well plates and induced to differentiate into adipocytes. The mature adipocytes were treated with 100 ng/ml IL-6 in DMEM/F12 and STAT3 specific blocker JSI-124 at different concentrations (0, 0.5, 1, 2, 5 and 10 μmol/L) for 48 h at 37°C in an environment with 5% CO₂. Total RNA was extracted, and RT-PCR was employed to detect the mRNA expressions of UCP-1, leptin, PPARγ and STAT3.

Statistical analysis

SPSS version 19.0 was employed for statistical analysis. Data are expressed as mean ± standard deviation ($\bar{X} \pm s$). One way analysis of variance was employed for comparisons among

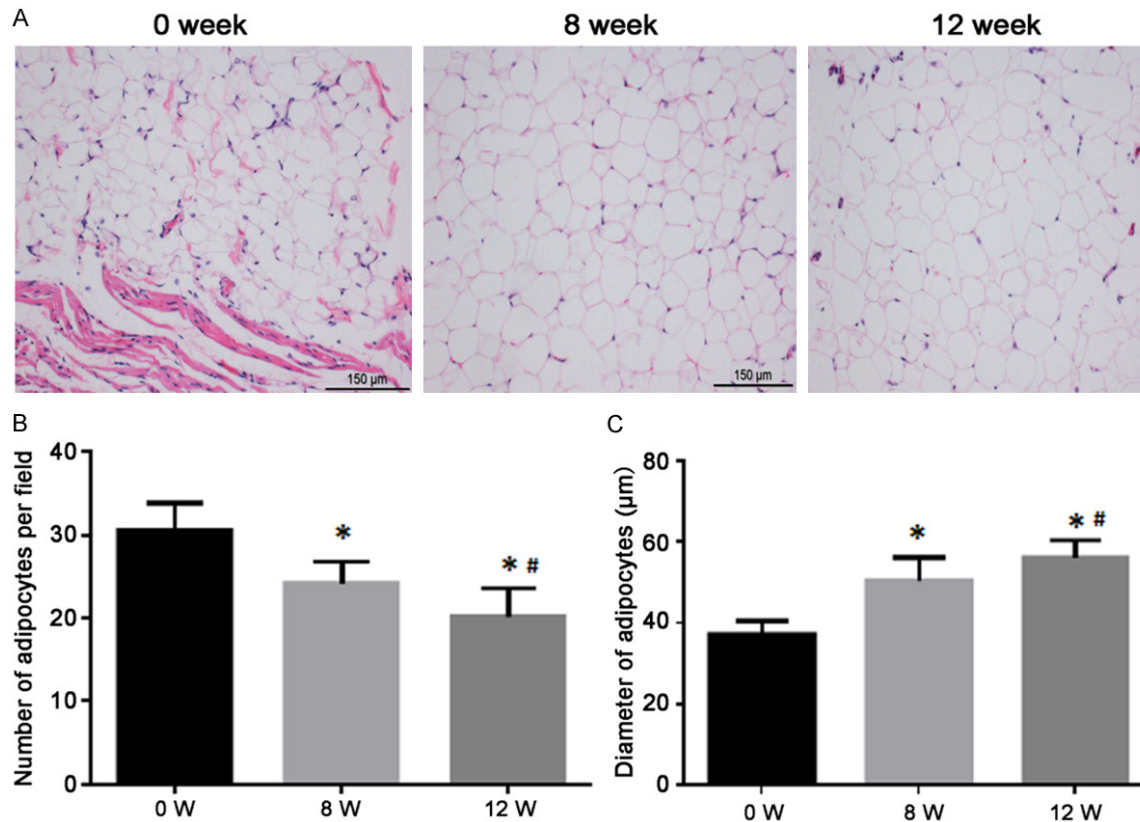


Figure 1. HE staining showed the number of adipocytes at 8 and 12 weeks reduced significantly and adipocyte volume increased when compared with those at week 0 ($P<0.05$). However, in control group, the number and volume of adipocytes remained stable during the experiment ($P>0.05$).

groups and t test for comparisons between groups. A value of $P<0.05$ was considered statistically significant.

Results

Blood lipids and plasma inflammatory cytokines

In experiment group, plasma TG, TC and LDL increased gradually over time, but HDL-C reduced progressively during the experiment ($P<0.05$). In control group, the blood lipids remained unchanged ($p>0.05$). In experiment group, plasma hs-CRP, IL-6, TNF- α , leptin, PAPP-A, sE-selectin and MMP-9 increased gradually, but adiponectin reduced progressively during the experiment ($P<0.05$). However, in the control group, the blood inflammatory cytokines remained stable ($P>0.05$) (**Table 2**).

Morphology of EAT and pathological of coronary plaques

Five fields were randomly selected for the evaluation of adipocytes. HE staining showed the number of adipocytes at 8 and 12 weeks

reduced significantly and adipocyte volume increased when compared with those at week 0 ($P<0.05$). However, in control group, the number and volume of adipocytes remained stable during the experiment ($P>0.05$) (**Figure 1**).

Masson staining indicated the plaque formation in the coronary artery at 8 and 12 weeks, suggesting that the atherosclerosis animal model was successfully established. At the same magnification, the area of plaques was enlarged gradually in experiment group (**Figure 2**).

TEM of adipose tissues

TEM showed the number of mitochondria with multiple cristae reduced in EAT at 8 and 12 weeks as compared to that at week 0, but it remained unchanged in control group (**Figure 3**).

Immunohistochemistry of adipose tissues

In experiment group, the expression of UCP-1 (a marker of BAT) reduced significantly, but that of leptin (a marker of WAT) reduced markedly;

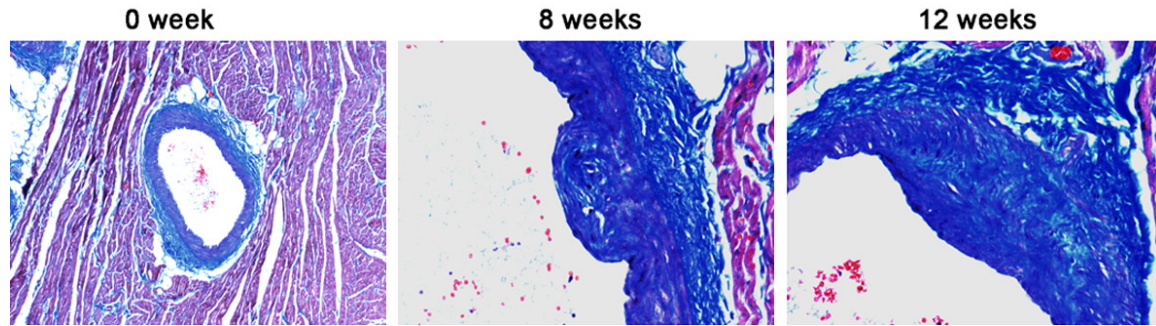


Figure 2. Masson staining indicated the plaque formation in the coronary artery at 8 and 12 weeks, suggesting that the atherosclerosis animal model was successfully established. At the same magnification, the area of plaques was enlarged gradually in experiment group.

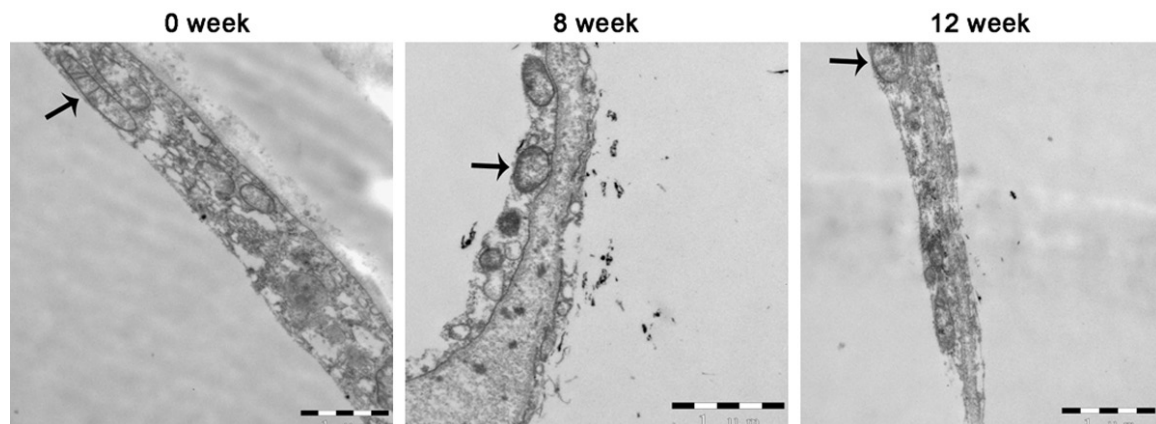


Figure 3. TEM showed the number of mitochondria with multiple cristae reduced in EAT at 8 and 12 weeks as compared to that at week 0, but it remained unchanged in control group.

CD31 expression reduced significantly, suggesting that the angiogenesis reduces; RAM11 expression (a marker of macrophage) increased, indicating that the inflammation increases in the adipose tissues ($P < 0.05$). However, these parameters remained stable in control group ($P > 0.05$) (**Figure 4**).

Expressions of genes specific to adipose tissues

At week 0, the mRNA expressions of UCP-1 and PPAR γ were comparable between EAT and sBAT, but significantly higher than in eWAT, and leptin mRNA expression in EAT and sBAT was markedly lower than in eWAT ($P < 0.05$). This indicates that EAT in healthy animals is BAT. In experiment group, the mRNA expressions of UCP-1 and PPAR- γ reduced gradually, but leptin mRNA expression increased gradually in EAT ($P < 0.05$). In control group, the mRNA expres-

sions of UCP-1, PPAR- γ and leptin remained unchanged ($P > 0.05$) (**Figure 5**).

Morphology of primary preadipocytes and mature adipocytes following induced differentiation

At several hours after adherence, cells were round and had difference sizes, and the nuclear/cytoplasmic ratio increased. At 2-4 days, cells began to stretch, most cells were spindle-shaped, some cells were polygonal and had clear three-dimensional structure, their nuclei were oval, and double nuclei were observed in some cells in mitosis. At 3-4 days, cells were monolayer-like, and these cells became oval (**Figure 6**). The growth of pre-adipocytes stopped after induction and began to differentiate. Under a light microscope, cells shrank, and lipid droplets were present around the nuclei and began to increase and enlarge. Finally, the

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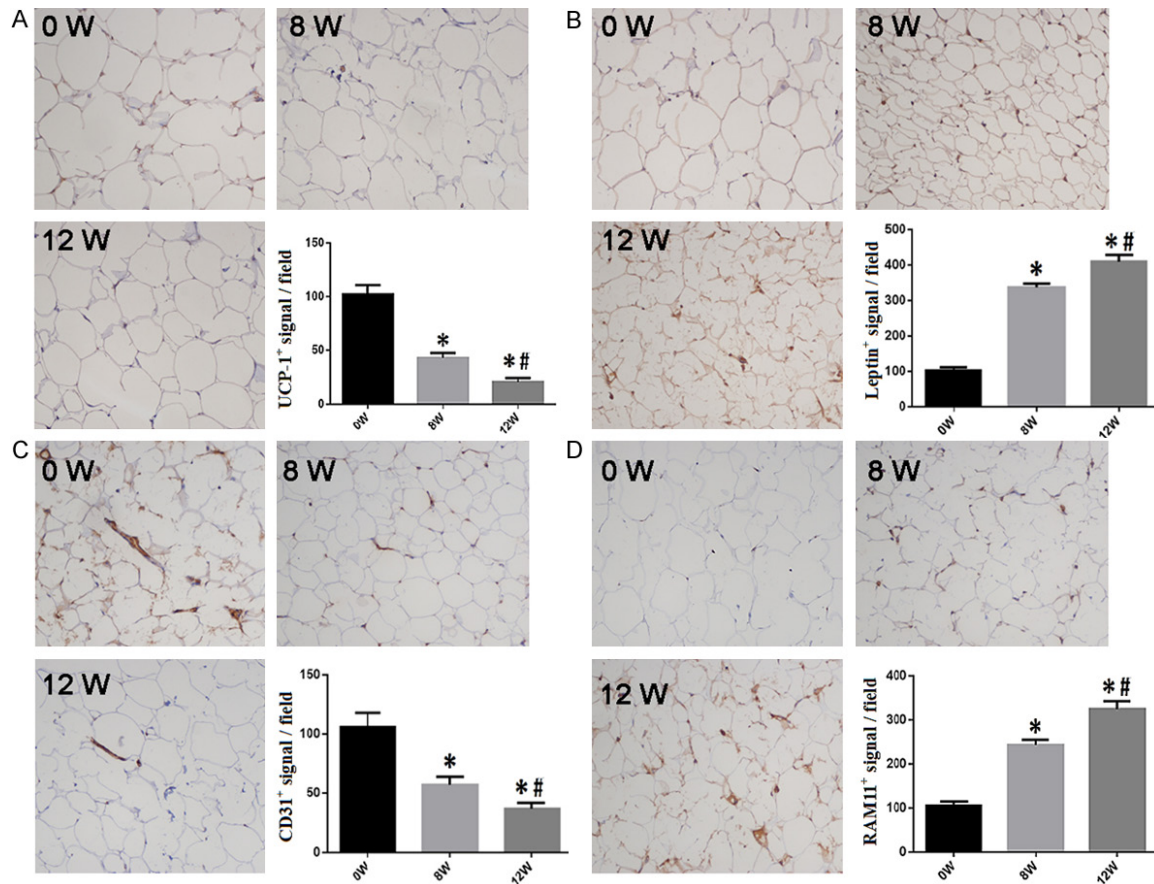


Figure 4. In experiment group, the expression of UCP-1 (a marker of BAT) reduced significantly, but that of leptin (a marker of WAT) reduced markedly; CD31 expression reduced significantly, suggesting that the angiogenesis reduces; RAM11 expression (a marker of macrophage) increased, indicating that the inflammation increases in the adipose tissues ($P < 0.05$). However, these parameters remained stable in control group ($P > 0.05$).

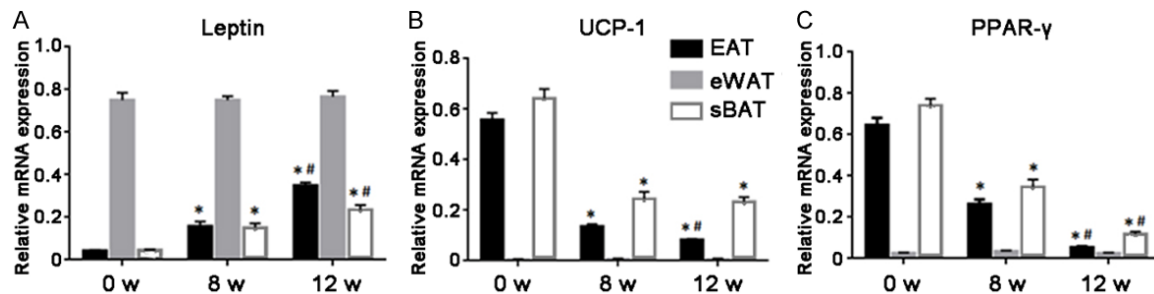


Figure 5. At week 0, the mRNA expressions of UCP-1 and PPAR γ were comparable between EAT and sBAT, but significantly higher than in eWAT, and leptin mRNA expression in EAT and sBAT was markedly lower than in eWAT ($P < 0.05$). In experiment group, the mRNA expressions of UCP-1 and PPAR- γ reduced gradually, but leptin mRNA expression increased gradually in EAT ($P < 0.05$). In control group, the mRNA expressions of UCP-1, PPAR- γ and leptin remained unchanged ($P > 0.05$).

lipid droplets were distributed in the whole cells, and the nuclei were squeezed into a thin rim at the periphery. At 6-8 days after induced differentiation, lipid droplets were present in

80% of cells, and most adipocytes had lipid droplets of different sizes. Oil red O staining showed red granules in BAT, which were found as lipid droplets (Figure 7).



Figure 6. At 3-4 days, cells were monolayer-like, and these cells became oval.

IL-6 induces the differentiation of brown adipocytes into white adipocytes

The mRNA expressions of UCP-1 and PPAR- γ reduced, but the mRNA expressions of leptin and STAT3 increased with the elevation of IL-6 dose. Significant differences were observed in the expressions of these genes when the dose of IL-6 was equal to or higher than 10 ng/ml ($P < 0.001$). These findings suggest that IL-6 may induce the phenotype conversion of EAT from BAT to WAT in a dose dependent manner (Figure 8).

STAT3 specific blocker JSI-124 inhibits the IL-6 induced conversion from BAT into WAT

After treatment with IL-6 at 100 ng/ml, the STAT3 specific blocker JSI-124 increased the mRNA expressions of UCP-1 and PPAR- γ in a dose dependent manner, but reduced the mRNA expressions of leptin and STAT3 in a dose dependent manner ($P < 0.01$). These results confirm that IL-6 may activate JAK-STAT3 to induce the conversion from BAT into WAT (Figure 9).

Discussion

Adipose tissues are the vital metabolic organ in human body and play an important role in the insulin sensitivity and energy balance. WAT and BAT have significant differences in the morphology, distribution and functions, which are as follows: (1) adipocytes in WAT have a single large lipid droplet which occupies a majority of cytoplasm; adipocytes in BAT have several small lipid droplets; (2) adipocytes in WAT have a few

mitochondria which possess a few cristae; adipocytes in BAT have a large amount of mitochondria with multiple cristae; (3) as compared to WAT, BAT has more blood vessels, and adipocytes in BAT have a large amount of cytochrome in the cytoplasm. That is why it is known as BAT. (4) WAT is mainly distributed in the subcutaneous, epididymal, mesenteric and perirenal tissues, but BAT is largely distributed in the neck, scapular and subscapularis tissues and tissues around the aorta and heart. BAT is helpful to improve the glucose balance and increase the energy consumption, which is beneficial for the metabolism. The major markers of BAT include UCP1, PRDM16, PGC-1 and CIDEA. WAT can secrete a lot of adipokines related to metabolic diseases. The markers of WAT include RIP140, resistin and leptin [4, 5]. It was proposed that there is no BAT in adults and BAT has no physiological importance. However, Truong et al [6] identified the active BAT in adults by using FDG-PET, which renews our understanding of BAT. In 2008, the source and differentiation of BAT had landmark breakthroughs [7]. To date, it has been confirmed that there is conversion from WAT to beige/brite and to BAT [8, 9]. Thus, to promote the BAT production or facilitate the conversion from WAT to BAT has been a focus in the therapy of obesity and relevant diseases [10, 11].

EAT refers to the adipose tissues between myocardium and visceral pericardium, which localize around the coronary artery. The functions of EAT are other than energy storage, and EAT mainly aggravates focal inflammation to involve the occurrence and development of coronary atherosclerosis [12]. A large amount of studies have confirmed that EAT may serve as an independent risk factor of CAD [13], and is closely related to the stability of plaques in coronary atherosclerosis [14]. Recent data from morphological, transcriptional profiling and proteomic analysis consistently demonstrated that EAT exhibits more characteristics of BAT than WAT [15], but high fat diet may promote the conversion from BAT around blood vessels into WAT and facilitate their release of pro-inflammatory cytokines (such as IL-6, IL-8 and MCTP-1) to induce coronary atherosclerosis [16]. In 2013, Sacks et al [17, 18] found the expressions of UCP-1, PRDM16 and PGC-1 (markers of BAT) increased significantly in EAT of adults with severe coronary atherosclerosis, and adult EAT

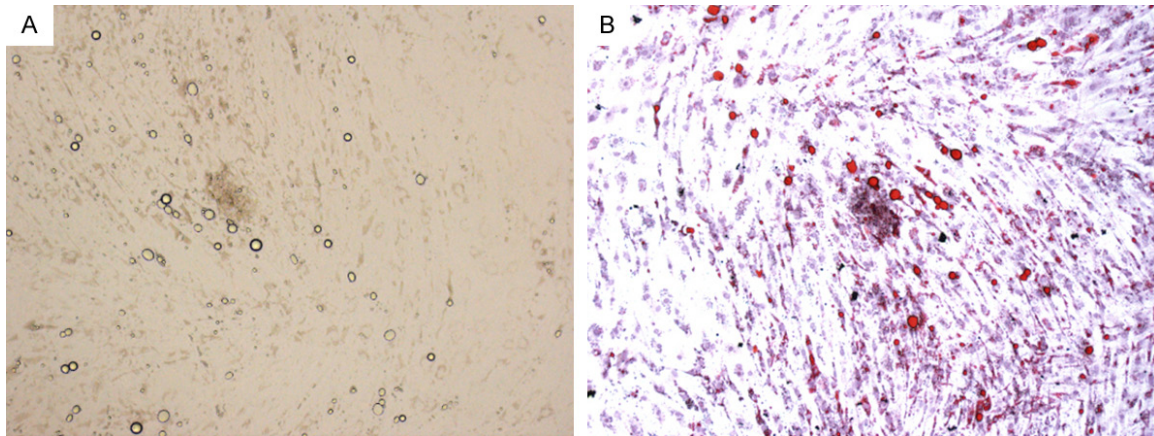


Figure 7. At 6-8 days after induced differentiation, lipid droplets were present in 80% of cells, and most adipocytes had lipid droplets of different sizes. Oil red O staining showed red granules in BAT, which were found as lipid droplets.

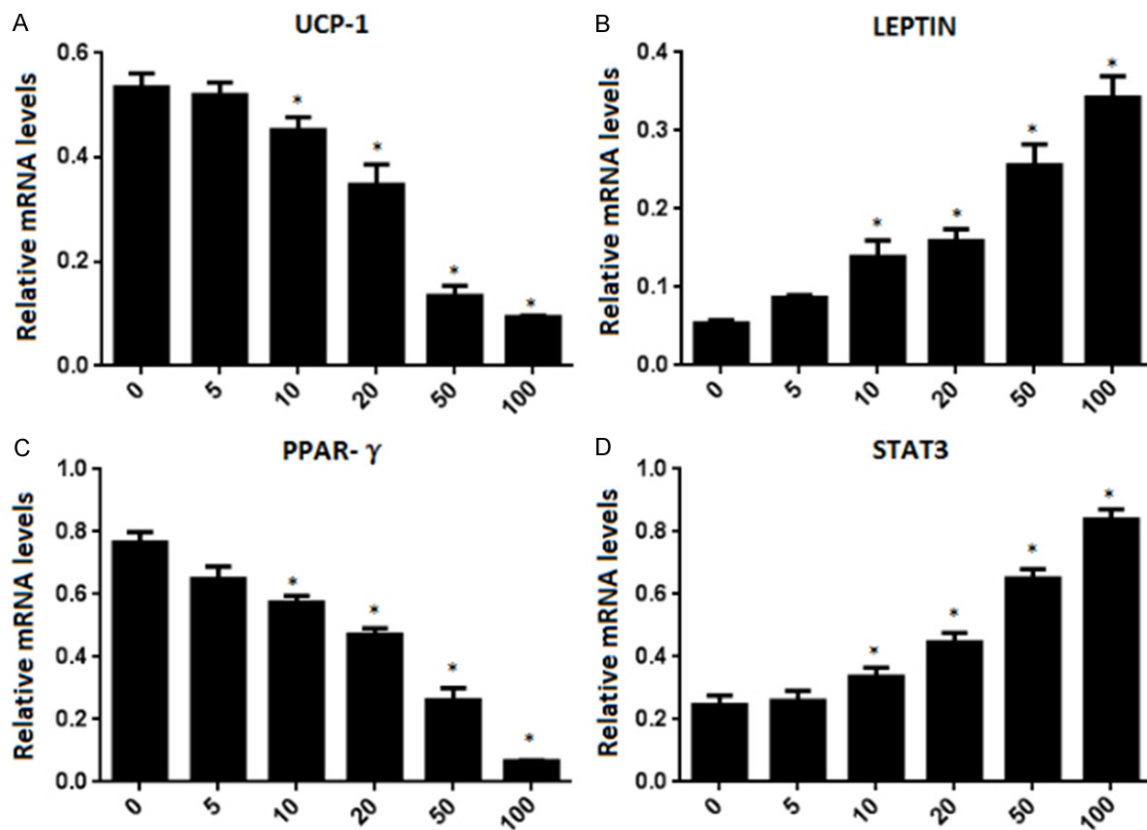


Figure 8. The mRNA expressions of UCP-1 and PPAR-γ reduced, but the mRNA expressions of leptin and STAT3 increased with the elevation of IL-6 dose. Significant differences were observed in the expressions of these genes when the dose of IL-6 was equal to or higher than 10 ng/ml ($P < 0.001$).

exhibited beige features in vitro. These findings imply that there is a conversion of EAT from BAT to WAT. Chechi et al [19] investigated 33 patients with CABG, and found the UCP1 expression in EAT was positively related to circulating

HDL-C. However, Dong et al [20] found cold exposure could increase the UCP1 expression, which promoted the growth and instability of atherosclerotic plaques. Thus, to elucidate the mechanism underlying the phenotype conver-

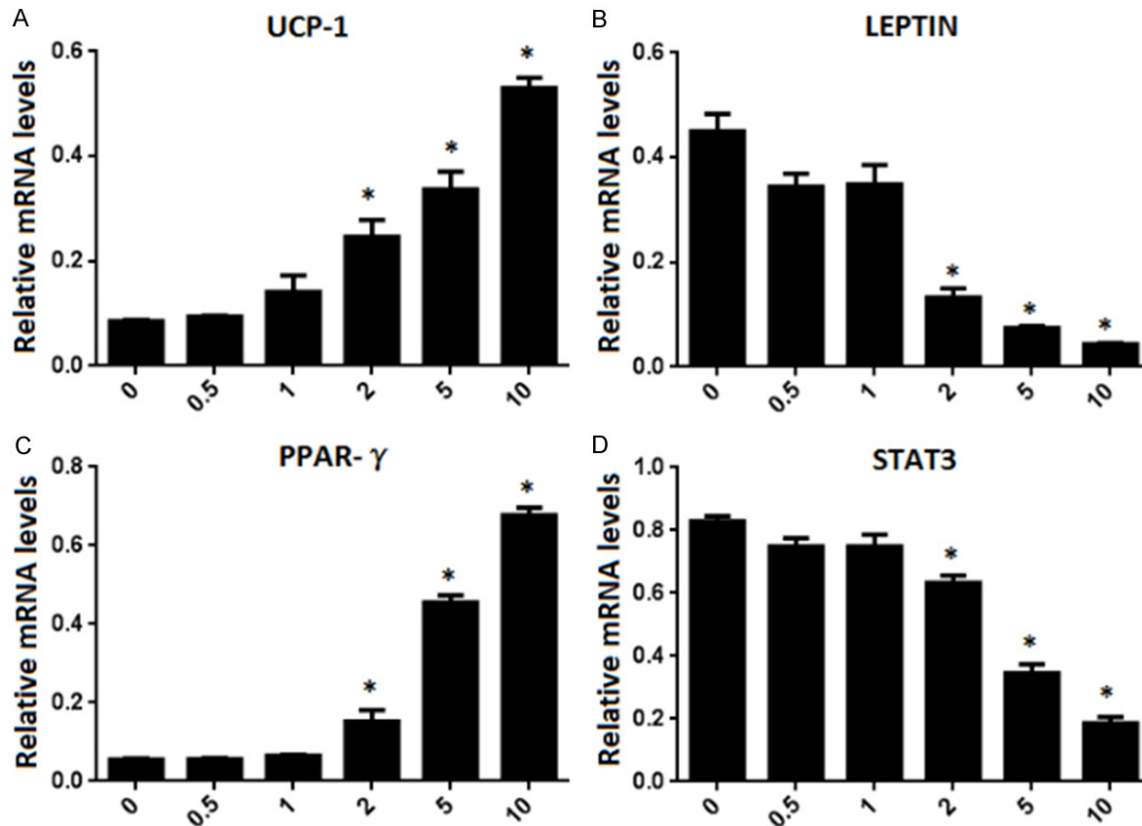


Figure 9. After treatment with IL-6 at 100 ng/ml, the STAT3 specific blocker JSI-124 increased the mRNA expressions of UCP-1 and PPAR- γ in a dose dependent manner, but reduced the mRNA expressions of leptin and STAT3 in a dose dependent manner ($P < 0.01$). These results confirm that IL-6 may activate JAK-STAT3 to induce the conversion from BAT into WAT.

sion of EAT is clinically important for the development of measures for the prevention and therapy of coronary atherosclerosis.

In the present study, our results showed the cell morphology was dense at week 0 in experiment group. With the progression of coronary atherosclerosis, HE staining confirmed the adipocyte volume was enlarged, and the number of adipocytes reduced in EAT; TEM showed the number of adipocytes with multiple cristae reduced. In our study, CD31 was used as a marker of angiogenesis. Immunohistochemistry showed the number of newly generated capillaries reduced significantly at 8 and 12 weeks. Both immunohistochemistry and RT-PCR confirmed the expressions of UCP-1 and PPAR- γ (specific marker of BAT) were the highest at week 0, but reduced gradually at weeks 8 and 12. However, the leptin expression (a marker of WAT) increased progressively over time. These findings suggest that EAT in healthy animals is

BAT and may convert from BAT into WAT during the progression of atherosclerosis.

Recent studies indicate that systemic inflammation may induce the conversion from BAT into WAT [21], and then WAT secret a lot of pro-inflammatory cytokines to aggravate local and systemic inflammation, which may result in or deteriorate obesity and metabolic diseases [16]. Leptin is synthesized and released by adipocytes in the WAT. Studies have shown that leptin is highly expressed in the macrophages of ruptured coronary atherosclerotic plaques and may serve as an independent risk factor of acute myocardial infarction [22]. E-selectin is a member of selectin family in adhesion molecules and mainly expressed on endothelial cells, and may serve as a marker of vascular endothelial cells [23]. sE-selectin is also related to the stability of coronary atherosclerotic plaques and can be used to evaluate the severity of CHD. MMP-9 and PAPP-A belong matrix

metalloproteinase superfamily and may impair the plaque structure, thin the fibrous cap and cause plaque rupture. PAPP-A may also induce the activation and chemotaxis of macrophages, increase foamy cells and cause the release of pro-inflammatory cytokines, to affect the formation and stability of plaques [24].

Our results showed, after feeding with high fat diet for 8 and 12 weeks, severe hypercholesterolemia was observed in rabbits, and pro-inflammatory cytokines (such as hs-CRP, IL-6, TNF- α , leptin, MMP-9, sE-selectin and PAPP-A) increased significantly, but adiponectin reduced markedly in the plasma. At the same time point, the number of macrophages in EAT around the plaques and leptin expression reduced significantly, suggesting that high fat diet may elevate the systemic inflammation. With the prolongation of high fat diet feeding, not only circulating cytokines reflecting early inflammation increased, but inflammatory cytokines reflecting the plaque vulnerability at late stage of atherosclerosis elevated. In addition, the inflammation of adipose tissues around the coronary artery deteriorated, suggesting that the local inflammation of EAT acts synergistically with circulating pro-inflammatory cytokines to involve the occurrence and development of coronary atherosclerosis and affect the stability of plaques, which further affects the occurrence of coronary events.

IL-6 is a pro-inflammatory cytokine with wide biological activities and mainly acts on JAK-STAT3 signaling pathway to transduce signals into the nucleus to induce target gene expression [25]. JAK-STAT signaling pathway is involved in the transduction of almost all the cytokine-related signals and plays an important regulatory role in the occurrence and development of diseases [26]. Banks et al [27] found leptin at a high level could induce SOCS-3 to block JAK-STAT3 signaling pathway, resulting in leptin resistance and subsequent obesity, which in turn deteriorates leptin resistance, leading to a vicious circulation. Zhang et al [28] found JAK-STAT3 signaling pathway played an important role in the early adipogenesis, but Wang et al [29] found JAK-STAT3 signaling pathway was able to regulate the differentiation of adipocytes. To date, no study has been conducted to investigate the role of JAK-STAT3 signaling pathway in the regulation of phenotype

conversion of EAT. In the present study, whether IL-6 activates JAK-STAT3 signaling pathway to induce the conversion from BAT into WAT was investigated. In the mature adipocytes from EAT, IL-6 at different concentrations was added. Results showed the mRNA expressions of UCP-1 and PPAR- γ reduced, but those of leptin and STAT3 increased gradually with the increase in IL-6 dose. This suggests that IL-6 may induce the conversion of BAT into WAT in a dose dependent manner. Then, JSI-124, a specific blocker of STAT3, was used in combination with IL-6 to treat these mature adipocytes. Results indicated that this conversion was blocked, suggesting that IL-6 is able to activate JAK-STAT3 to induce the conversion of BAT into WAT.

Conclusion

The EAT in healthy animals is BAT. During the progression of coronary atherosclerosis, there is a phenotype conversion of EAT from BAT into WAT. Systemic inflammation status may facilitate the conversion of EAT into WAT. IL-6 may activate JAK-STAT3 signaling pathway to induce the conversion from BAT into WAT. Besides circulating inflammation, local inflammation of EAT is also involved in or promotes the occurrence and development of coronary atherosclerosis. BAT has been a focus in the field of obesity related metabolic diseases and a target in the development of weight loss drugs. However, the amount of BAT is small, which limits this development. To inhibit the conversion of EAT from BAT into WAT may facilitate the energy consumption, improve the glucose and lipid metabolism, and attenuate the coronary atherosclerosis. Our findings provide new clues for the future therapy of metabolic diseases including CHD, but these were from animal models. Whether this therapy is applicable in the early prevention of CHD in clinical practice and in the induction of conversion of BAT into WAT for the therapy of CHD is required to be studied in depth.

Acknowledgements

The study was supported by the "Six Peaks of The Talented in Jiangsu Province".

Disclosure of conflict of interest

None.

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